AWARD NUMBER: W81XWH-16-1-0230

TITLE: Control of Lung Inflammation by Microbiome and Peptidoglycan Recognition Protein

PRINCIPAL INVESTIGATOR: Roman Dziarski

RECIPIENT: **Trustess of** Indiana University
Gary, IN 46408

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TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2017	Annual	1 Jul 2016 - 30 Jun 2017

4. TITLE AND SUBTITLE

Control of Lung Inflammation by Microbiome and Peptidoglycan Recognition Protein

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0230

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Roman Dziarski

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
TRUSTEES OF INDIANA UNIVERSITY, INDIANAPOLIS, IN 46202

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project is using a mouse model of experimentally-induced asthma to test two hypotheses: (i) that respiratory and intestinal microbiomes control sensitivity to asthma; and (ii) that this microbiome is controlled by antibacterial innate immunity protein, Peptidoglycan Recognition Protein 1 (*Pglyrp1*). Microflora was depleted in mice with antibiotics and pregnant females and their pups were colonized with microfloras from wild-type mice or from *Pglyrp1*-deficient mice. These pups were then sensitized with house dust mite allergen to induce asthma. Mice colonized with microfloras from wild-type or *Pglyrp*-deficient mice had similar severity of asthma and lung inflammation, as measured by lung resistance test and extent of infiltration with inflammatory cells. By contrast, germ-free mice (completely devoid of microflora), similarly colonized with microbiomes from *Pglyrp1*$^{-/-}$ mice and sensitized, had significantly less severe asthma and lung inflammation than germ-free mice colonized with microbiomes from wild-type mice, as measured by lung resistance test and extent of infiltration with inflammatory cells. These results indicate that microbiome significantly affects sensitivity to asthma and lung inflammation and that microbiome from *Pglyrp1*-deficient mice reduces allergic inflammatory response in the lungs compared with microbiome from wild-type mice.

15. SUBJECT TERMS
Asthma, Acute Lung Injury, Microbiome, Innate Immunity, Peptidoglycan Recognition Protein 1, Pglyrp1

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

This project is testing an emerging idea that the abundance and the composition of respiratory and intestinal microbiomes controls sensitivity to asthma and that one of the important host factors that controls the abundance and composition of microbiome is antibacterial innate immunity protein, **Peptidoglycan Recognition Protein 1** (***Pglyrp1***). The role of *Pglyrp1*-controlled respiratory and intestinal tract microfloras in the sensitivity to asthma and lung and airways inflammation is being tested using a mouse model of experimentally induced asthma.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Asthma, Acute Lung Injury, Microbiome, Innate Immunity, Peptidoglycan Recognition Protein 1, Pglyrp1

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1: Obtain IUSM-NW IACUC and DoD ACURO Animal Care and Use Application reviews and approvals	**Month 1**	**100% completed**
Milestone(s) Achieved: IUSM-NW IACUC and DoD ACURO Animal Care and Use Application approvals obtained		Completed
<u>Specific Aim 1</u>: Identify *Pglyrp1*-controlled microflora in respiratory and intestinal tract microbiomes		
Major Task 2: Collect respiratory tract and intestinal microbiome samples from WT and *Pglyrp1*$^{-/-}$ mice, isolate bacterial DNA, sequence bacterial 16R rRNA genes, and analyze composition of microbiomes	**Months 1–3**	**10% completed**
Milestone(s) Achieved: Identification of bacterial diversity in the respiratory and intestinal tracts of WT and *Pglyrp1*$^{-/-}$ mice, and identification of significant differences in these bacteria between *Pglyrp1*$^{-/-}$ and WT mice		Subtask 1 completed, Subtasks 2–4 pending
<u>Specific Aim 2</u>: Determine the role of *Pglyrp1*-controlled respiratory and intestinal tract microfloras in the changed sensitivity of mice to asthma and lung and airways inflammation		

Major Task 3: Determine the role of the entire respiratory microbiome and intestinal microbiome of WT and *Pglyrp1*$^{-/-}$ mice in sensitivity to asthma	**Months 4–10**	**90% completed**
Milestone(s) Achieved: Identification of the ability of respiratory and/or intestinal microflora from WT and *Pglyrp1*$^{-/-}$ mice to control sensitivity to asthma		Subtasks 1–3 100% completed, Subtask 4 50% completed
Major Task 4: Determine the role of microflora that is more abundant in *Pglyrp1*$^{-/-}$ than in WT mice in controlling sensitivity to asthma	**Months 11–14**	**Pending**
Milestone(s) Achieved: Identification of the ability of bacteria that are more abundant in *Pglyrp1*$^{-/-}$ mice than in WT mice to control sensitivity to asthma		Pending
Major Task 5: Determine the role of microflora that is more abundant in WT than in *Pglyrp1*$^{-/-}$ mice in controlling sensitivity to asthma	**Months 15–18**	**Pending**
Milestone(s) Achieved: Identification of the ability of bacteria that are more abundant in WT mice than in *Pglyrp1*$^{-/-}$ mice to control sensitivity to asthma		Pending

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task 1: IUSM-NW IACUC and DoD ACURO Animal Care and Use Application approvals obtained.

Specific Aim 1: Identify *Pglyrp1*-controlled microflora in respiratory and intestinal tract microbiomes

Major Task 2: Collect respiratory tract and intestinal microbiome samples from WT and *Pglyrp1*$^{-/-}$ mice, isolate bacterial DNA, sequence bacterial 16R rRNA genes, and analyze composition of microbiomes

Subtask 1: Collect respiratory tract and intestinal microbiome samples from WT and *Pglyrp1*$^{-/-}$ mice.

The samples were collected and persevered.

Subtasks 2–4: Isolate bacterial DNA from respiratory tract and intestinal microbiome samples from WT and *Pglyrp1*$^{-/-}$ mice; Perform pyrosequencing of bacterial 16R rRNA genes in DNA samples obtained in subtask 2 and assign sequences to taxonomic units; Compare diversity of operational taxonomic units (OTUs), species, genera, families, orders, classes, and phyla in respiratory and intestinal microbiomes and determine significant differences between WT and *Pglyrp1*$^{-/-}$ mice; identify species significantly increased in WT and *Pglyrp1*$^{-/-}$ mice.

These Subtasks will be performed in the second year of the project, after completing Major Task 3. The reason for performing Subtasks 2–4 after completing Major Task 3 is that we want to make sure that the microbiomes we are sequencing and analyzing have the capacity to modulate the sensitivity to asthma, which will be determined in Major Task 3.

Specific Aim 2: Determine the role of *Pglyrp1*-controlled respiratory and intestinal tract microfloras in the changed sensitivity of mice to asthma and lung and airways inflammation

Major Task 3: Determine the role of the entire respiratory microbiome and intestinal microbiome of WT and *Pglyrp1*$^{-/-}$ mice in sensitivity to asthma

Subtask 1: Collect and preserve respiratory and intestinal microflora from WT and *Pglyrp1*$^{-/-}$ mice. We collected the microflora samples, preserved them, and used them in Subtask 2.

Subtask 2: Colonize germ-free or antibiotic-treated mice with respiratory microflora, intestinal microflora, or both microfloras from WT or *Pglyrp1*$^{-/-}$ mice.
Subtask 3: Sensitize mice from subtask 2 with HDM allergen and induce asthmatic inflammatory response to HDM.
Subtask 4: Measure the severity of asthma and lung and airways inflammation in mice from subtask 3.

In Experiment 1, we depleted microbiomes in conventional WT BALB/c male and female mice using 3-week long antibiotic treatment, we mated these mice, and then we colonized pregnant females with respiratory and intestinal microfloras from WT or from *Pglyrp1*$^{-/-}$ mice. We continued re-colonizing nursing mothers until weaning, and then we continued re-colonizing pups after weaning throughout the entire experiment. At 6 weeks of age we began intranasal sensitization of the pups with house dust mite (HDM) allergen, which we continued for 5 weeks to induce chronic asthma-like lung inflammation. We then measured the severity of asthma and lung and airways inflammation using lung function and histo-pathologic and immunologic tests (Fig. 1).

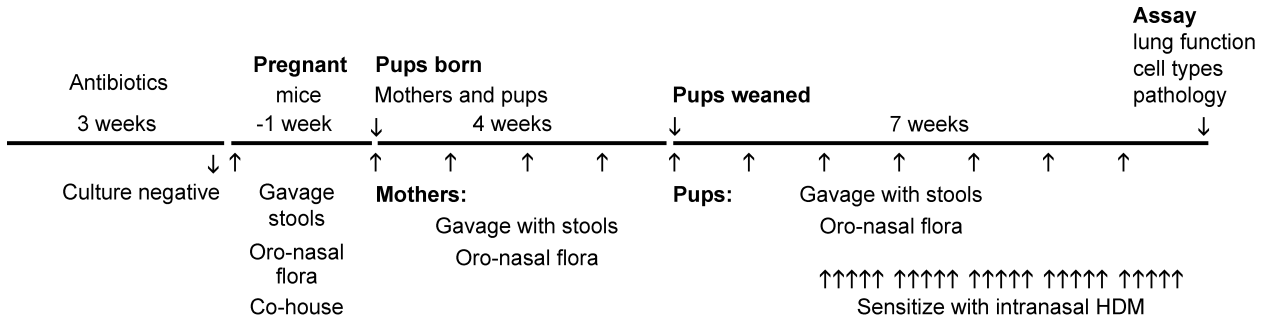

Fig. 1. Experimental timeline for depletion of microflora in BALB/c mice with oral antibiotics and colonization of pregnant mice and pups with respiratory and intestinal microfloras from WT or from *Pglyrp1*$^{-/-}$ mice, sensitization of pups with HDM, and asthma assays.

Experiment 1 results: Both groups of mice (colonized with microfloras from WT or from *Pglyrp1*$^{-/-}$ mice) had similar severity of asthma and lung inflammation, as measured by lung resistance test (Fig. 2) and extent of infiltration with inflammatory cells (Fig. 3). These results could be interpreted in two ways: (i) the effect of microbiome from *Pglyrp1*$^{-/-}$ mice could not be demonstrated because antibiotics did not sufficiently deplete the microflora in the parents and the original microflora came back and became dominant over the colonized microflora after antibiotic treatment was stopped; or (ii) microbiome from *Pglyrp1*$^{-/-}$ mice did get established, but had no effect on the severity of asthma and lung inflammation. To distinguish between these two possibilities, Experiment 2 was then performed.

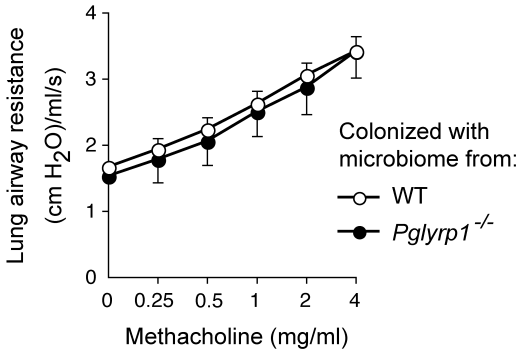

Fig. 2. Lung airway resistance in response to methacholine in microflora-depleted BALB/c mice colonized with respiratory and intestinal microfloras from WT or from *Pglyrp1*$^{-/-}$ mice and sensitized with HDM, as shown in Fig. 1. The results are means ± SEM of 11 mice per group. The differences between WT and *Pglyrp1*$^{-/-}$ groups were not statistically significant.

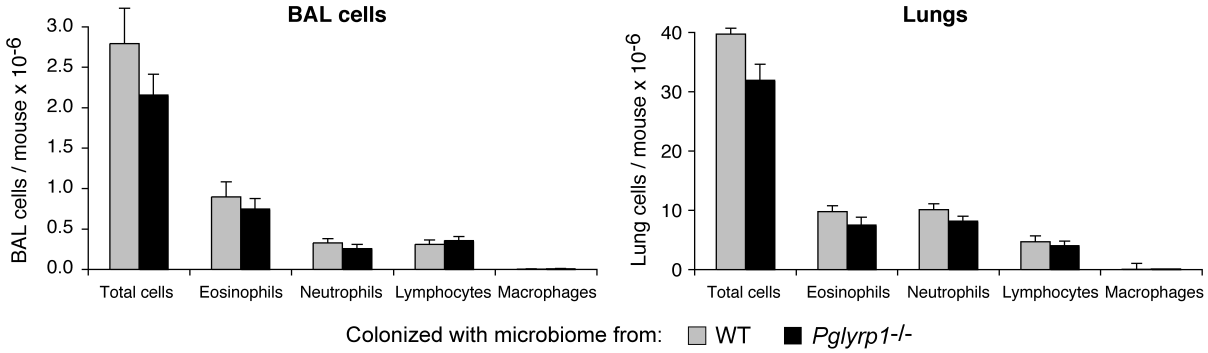

Fig. 3. Inflammatory cells in bronchoalveolar lavage (BAL) fluid and lungs in microflora-depleted BALB/c mice colonized with respiratory and intestinal microfloras from WT or from *Pglyrp1*$^{-/-}$ mice and sensitized with HDM, as shown in Fig. 1. The results are means ± SEM of 11 mice per group. The differences between WT and *Pglyrp1*$^{-/-}$ groups were not statistically significant.

Experiment 2 was performed the same way as Experiment 1, but Germ-free WT Swiss-Webster mice were used instead of antibiotic-treated conventional mice, to start with mice completely devoid of microbiome and to eliminate the possibility of incomplete depletion and re-emergence of the original microbiome (Fig. 4).

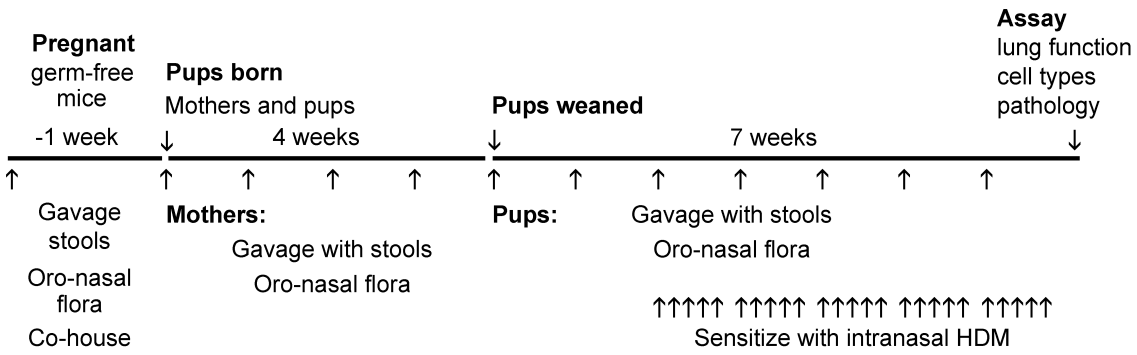

Fig. 4. Experimental timeline for colonization of germ-free pregnant mice and pups with respiratory and intestinal microfloras from WT or from *Pglyrp1*$^{-/-}$ mice, sensitization of pups with HDM, and asthma assays.

Experiment 2 results: Germ-free mice colonized with microbiomes from *Pglyrp1*$^{-/-}$ mice had significantly less severe asthma and lung inflammation than Germ-free mice colonized with microbiomes from WT mice, as measured by lung resistance test (Fig. 5) and extent of infiltration with inflammatory cells (Fig. 6). These data indicate that microbiome significantly affects sensitivity to asthma and lung inflammation and that microbiome from *Pglyrp1*$^{-/-}$ mice reduces allergic inflammatory response in the lungs compared with microbiome from WT mice.

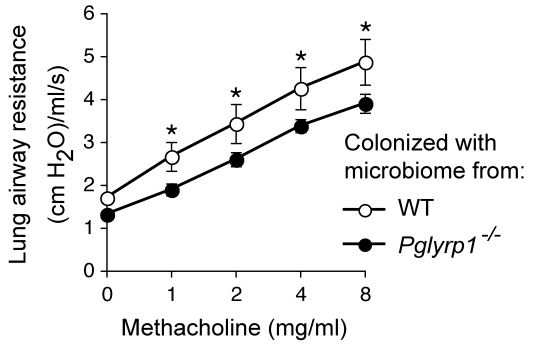

Fig. 5. Lung airway resistance in response to methacholine in germ-free Swiss-Webster mice colonized with respiratory and intestinal microfloras from WT or from *Pglyrp1*$^{-/-}$ mice and sensitized with HDM, as shown in Fig. 4. The results are means ± SEM of 12 mice per group; * $P < 0.05$ for WT versus *Pglyrp1*$^{-/-}$ groups (*t*-test).

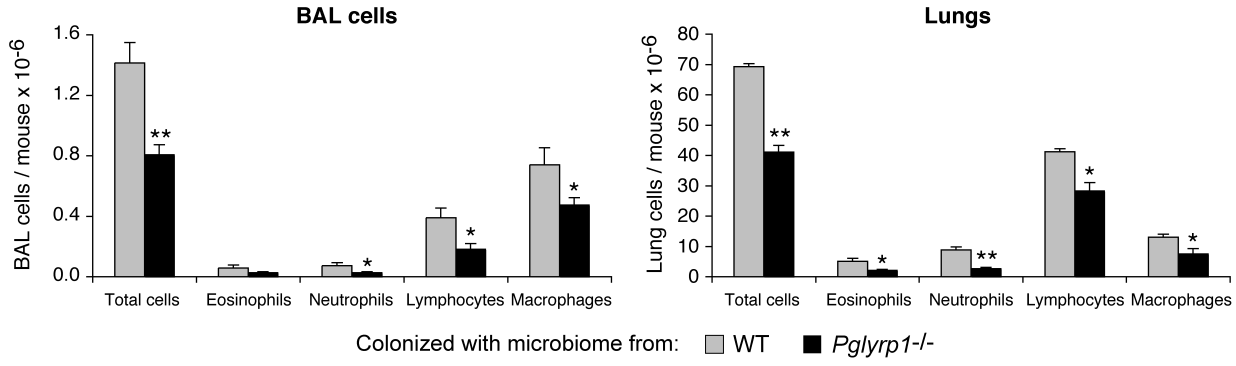

Fig. 6. Inflammatory cells in BAL fluid and lungs in germ-free Swiss-Webster mice colonized with respiratory and intestinal microfloras from WT or from *Pglyrp1*$^{-/-}$ mice and sensitized with HDM, as shown in Fig. 4. The results are means ± SEM of 12 mice per group; * $P < 0.05$, ** $P < 0.001$, for WT versus *Pglyrp1*$^{-/-}$ groups (*t*-test).

We are still analyzing other samples and data from Experiment 2, to further characterize the type of inflammatory response in these mice affected by colonization with these two types of microbiomes. Our data available so far indicate that although the colonized Swiss-Webster mice become sensitized with HDM and show inflammatory response in the lungs, these mice do not develop typical allergic inflammation seen in human asthma and in experimental asthma in BALB/c mice, characterized by high eosinophilic response. This difference is probably due to genetic differences between Swiss-Webster and BALB/c mice. We will confirm these results by analyzing lung histopathology of mice from Experiment 2. BALB/c mice are a preferred model of human asthma, because similar to humans with asthma, BALB/c mice develop an allergic eosinophilic response to respiratory HDM sensitization. For this reason, to better mimic human asthma, in the second year of this project we will repeat Experiment 2 using Germ-free BALB/c mice, instead of Swiss-Webster mice. Please note that Germ-free BALB/c mice were not available when we started this project and for this reason we had to use Germ-free Swiss-Webster mice, but now Germ-free BALB/c mice became commercially available and we are planning to use them. This planned repetition of Experiment 2 will reveal the effect of microbiome from *Pglyrp1*$^{-/-}$ mice in a BALB/c asthma model that highly resembles human asthma and is more suitable for the goals of this project.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."
Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the second year, we plan to finish Major Task 2 and Major Task 3, and perform and complete Major Task 4 and Major Task 5, as outlined above under the "Goals of the project" and described in detail in our original SOW. Please note that some of the subtasks that were originally planned for year 1 of the project will be now performed in year 2, because of the need to hire new personnel for this project.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The **short-term impact** of our project so far is that our data support the hypothesis that *Pglyrp1* gene controls the composition of respiratory and/or intestinal microflora, and that this microflora influences the sensitivity to asthma and lung inflammation. This conclusion will be further verified in the second year of this project, along with an attempt to identify the groups of bacterial species responsible for this effect.

The **long-term impact** of this project (once completed) will be future application of these results for the development of new prevention and treatment methods for asthma and other inflammatory diseases. These methods will involve modulating expression or activity of innate immunity molecules that control microbiome, or re-balancing respiratory and/or intestinal microflora to maximize its beneficial effect, by increasing asthma-protective microflora and eradicating asthma-promoting microflora.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*

- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Some of the subtasks that were originally planned for year 1 of the project will be now performed in year 2. This delay was caused by the need to search for and to hire new personnel for the project. Also, we will repeat Experiment 2 in Major Task 3, as described in Section 3 Accomplishments.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

A delay in performing some of the subtasks was caused by the need to find and hire new appropriately qualified research personnel to perform the experiments and the need to obtain their visas. One scientist could only work for three months and another scientist for six months, and we are now waiting to complete the visa paperwork for their replacement. Once the new scientist arrives, we will complete all the tasks during year 2, but we will likely seek a no-cost extension.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

No significant changes.

Significant changes in use or care of vertebrate animals.

No significant changes.

Significant changes in use of biohazards and/or select agents

No significant changes.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to Report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time*

conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report.

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Presentation by Roman Dziarski, titled "PGRPs – antibacterial proteins that regulate microbiome and inflammation", at the Symposium on "Microbiome, Bacterial Peptidoglycan and the Central Nervous System" at Karolinska Institute, Stockholm, Sweden, April 6, 2017.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the

understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Name:	Roman Dziarski
Project Role:	Principal Investigator
Nearest person month worked:	1
Contribution to Project:	Dr. Dziarski planned, designed, and performed the experiments, and analyzed the results.
Name:	Des R. Kashyap
Project Role:	Postdoctoral Fellow
Nearest person month worked:	3
Contribution to Project:	Dr. Kashyap performed the experiments on Major Tasks 2 and 3.
Name:	María D. Juárez-Rodríguez
Project Role:	Postdoctoral Fellow
Nearest person month worked:	6
Contribution to Project:	Dr. Juárez-Rodríguez performed the experiments on Major Task 3.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Current Active Support:

Source and Project Number: NIH 1R01AI120962-01
Principal Investigator: Dziarski, Roman
Title of Project: Antibacterial activity of peptidoglycan recognition proteins
Percent Effort: 20%
Dates of Project: 07/06/2016 – 06/30/2020
Total Direct Costs: $1,000,000
Total Costs: $1, 571,250
Goals: The major goal of this project is to determine the mechanism of bactericidal activity of peptidoglycan recognition proteins (PGRP).
Specific Aims:

1. We will determine that each of the 13 proposed events actually happens during PGRP killing of bacteria.
2. We will determine which of these 13 proposed events participate in PGRP-induced killing and which in bacterial defense against killing, or which are a consequence of killing.
3. We will determine the sequence of these 13 proposed events in PGRP-induced killing and which events are sequential and which parallel.

Overlap: There is no overlap with this DoD project.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc.,*

available to project staff);

- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

No Appendices.